Remarks

Rejections under 35 U.S.C. § 103

Claims 1-32, 67-75, and 78-100 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Baldarelli et al. (6,015,714), hereinafter "Baldarelli" in view of Lizardi (6,632,609), hereinafter "Lizardi". Baldarelli discloses a method of nucleic acid sequencing that involves passing a nucleic acid strand through a pore or channel. Lizardi teaches compositions and methods for amplification and multiplex detection of molecules of interest involving rolling circle amplification.

As set forth in MPEP §706.02(j), Contents of a 35 U.S.C. §103 Rejection, "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." See *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The Examiner states that "Lizardi discloses rolling circle amplification involving circular template...to make a long DNA molecule containing multiple repeats of sequences complementary to the circular template (or open circle probe)...The modified nucleotides caged in the probe are modified adenosine, modified thymidine, modified guanosine, and modified cytosine." The Examiner then provides an asserted motivation to combine. Applicants submit that the Examiner's reasoning utilizes hindsight, does not accurately reflect the teachings of Lizardi, and would not result in the claimed invention for each of the reasons set forth below.

Firstly, the Examiner's asserted motivation to combine utilizes hindsight, is in part irrelevant, and confuses different aspects of Lizardi. The Examiner suggests that "one of ordinary skill in the art would have been motivated to modify the method of Baldarelli et al. by applying the circular template and modified base as taught by Lizardi et al because as taught by Lizardi, rolling circle amplification results in a large amplification of the circularized probe sequences...(See col. 3, lines 16-20) and the modified nucleotide in the probe protect the 3' hydroxyl and render the degenerate probes incapable of participating in DNA polymerase extension (See col. 78, lines 11-16) to produce different lengths of tandem repeat sequences (See

col. 22, lines 2-6). It is unclear whether the Examiner intends here to set forth two possible motivations to combine, i.e., (i) rolling circle amplification results in a large amplification of the circularized probe sequences; and (ii) the modified nucleotide in the probe protect (*sic*) the 3' hydroxyl and render the degenerate probes incapable of participating in DNA polymerase extension, or whether the Examiner believes that these two aspects are related to one another. In any case, neither (i) nor (ii) provides motivation to combine, and there is no logical relationship between (i) and (ii).

With respect to (i), Applicants submit that the Examiner is using hindsight to suggest that Lizardi's teaching that rolling circle amplification results in a large amplification of the probe sequences provides motivation to combine. Rolling circle amplification as taught by Lizardi results in a molecule that contains multiple copies of a nucleic acid of interest. The teaching that it is advantageous to perform nanopore sequencing using a template comprising multiple copies of a nucleic acid of interest is found in the instant invention (see, e.g., paragraph 8, p. 12, line 19 – p. 13, line 6) and is not found in Baldarelli. Baldarelli states that according to his invention, "Individual molecules in a population may be characterized in rapid succession." Baldarelli does not teach or suggest that it would be desirable to sequence a molecule that contained multiple amplified copies of a single nucleic acid of interest. If anything Baldarelli teaches away from doing so since he indicates that his method is *rapid*. Other factors being equal, sequencing a template that contains multiple copies of a nucleic acid of interest would be expected take longer than sequencing a template containing only a single copy of the nucleic acid of interest. In any case, even if there was motivation to use a template produced by rolling circle amplification as taught by Lizardi in the method of Baldarelli, this alone would not teach the claimed invention, which requires a nucleic acid molecule containing modified nucleotides that reduce secondary structure. The Examiner appears to suggest that there is motivation to use the modified nucleotides taught by Lizardi to produce a template using rolling circle amplification. Applicants respectfully disagree and submit that even if such motivation existed, it would not result in the claimed invention.

Applicants note that the modified nucleotides taught by Lizardi, referred to as "caged nucleotides", contain a "removable blocking group which prevents the 3' hydroxyl from participating in nucleotide addition and ligation reactions" (col. 21, lines 33-36). Applicants submit that (a) by juxtaposing references to circularized probe sequences, degenerate probes, and

the production of different lengths of tandem repeat sequences, the Examiner is confusing different aspects of Lizardi's invention; and (b) the fact that a modified nucleotide in the probe protects the 3' hydroxyl and renders the degenerate probes incapable of participating in DNA polymerase extension is irrelevant, provides no motivation to combine, and actually demonstrates that a template produced using modified nucleotides as taught by Lizardi could contain at most one modified nucleotide, i.e., at the 3' end of the template.

Lizardi teaches several different types of probes and primers, some of which may comprise a modified nucleotide. The "circularized probe sequences" are nucleic acids of interest that are to be detected using his methods. They do not contain caged nucleotides. The "degenerate probes" described in col. 78 and also at col. 56, lines 47-48 are used, e.g., for primer extension sequencing. They are not part of the template produced by rolling circle amplification, and their features are irrelevant for purposes of establishing motivation to combine. There is no indication that it would be desirable to protect the 3' end of a template to be sequenced according to the claimed methods to prevent them from participating in DNA polymerase extension.

When Lizardi discusses the use of oligonucleotides containing caged nucleotides for production of different lengths of tandem repeat sequences, he is referring to replication primers, not degenerate probes as confusingly suggested by the Examiner (see, e.g., col. 21, line 63- col. 22, line 6). Furthermore, as taught by Lizardi at col. 33, lines 20-30, when replication primers containing caged nucleotides are used in rolling circle amplification, "The caged rolling circle replication primer will not support rolling circle replication until the cage structure is removed." (col. 33, lines 23-25). The cage structure must clearly be removed, because by design it is intended to prevent extension. Thus the template produced by rolling circle replication as taught by Lizardi would not contain caged nucleotides even if replication primers containing such nucleotides were used.

In fact, it is evident wherever Lizardi discusses caged nucleotides that regardless of the context in which the caged nucleotides are used, e.g., in replication primers, interrogation probes, or degenerate probes, they must be removed before an oligonucleotide containing them at its 3' end can be extended or incorporated into a larger structure by polymerization or extension. In addition to the excerpts referenced above, see, e.g., col. 18, lines 35-44; col. 55, lines 44-55; and Example 10 (cols. 74-77). Thus the fact that the modified nucleotide protects the 3' hydroxyl, asserted by the Examiner to provide motivation to combine, in fact renders the nucleotides

unusable in rolling circle replication in their modified form. Once the cage structure has been removed, the nucleotide is no longer modified. At most a template could contain one such modified nucleotide, i.e., at its 3' end. In fact the templates produced according to Lizardi's method would not contain even a single modified nucleotide. Furthermore, there is no evidence to suggest that a template with a single caged nucleotide at its 3' end would have reduced secondary structure. Applicant notes that the modification occurs at the 3' position of the sugar moiety rather than in the base portion of the nucleotide. Although the intended use does not render patentable weight, as indicated by the Examiner, the result must still meet the limitation of the claims, i.e., the modified nucleotide must reduce secondary structure in the nucleic acid molecule.

In summary, Applicant submits that none of the three criteria necessary for a *prima facie* case of obviousness have been met because (i) there is no motivation to combine the teachings of Lizardi and Baldarelli; (ii) there would be no reasonable expectation of success since the modified nucleotides taught by Lizardi cannot be extended by polymerization or ligation until the modification has been removed and it would thus not be possible to use rolling circle amplification to create a nucleic acid molecule containing more than one such nucleotide (at its 3' end); (iii) given that any nucleic acid molecule synthesized by rolling circle amplification using the modified nucleotides of Lizardi could contain at most one modified nucleotide it is highly unlikely that such a nucleic acid molecule would have reduced secondary structure as required by the instant claims, and thus the combination of Lizardi and Baldarelli does not teach the claimed invention. Given that not a single criterion for a *prima facie* case of obviousness is met, Applicant respectfully requests withdrawal of the rejection.

Claims 33-34 and 76-77 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Baldarelli in view of Lizardi as applied to claims 1-32, 67-75, and 78-100, and further in view of Ross (6,175,004), hereinafter "Ross". The Examiner points out that Ross discloses an oligonucleotide that contains 2-aminoadenosine and "like moieties" and states that there would have been motivation to modify the method of Baldarelli by applying a "nucleic acid molecule containing 2-aminoadenosine because the nucleic acid molecule containing 2-aminoadenosine provides stronger hybridization to their target sequences." The Examiner does not explain how the teachings of Lizardi are applied here. The Examiner also does not explain how the fact that

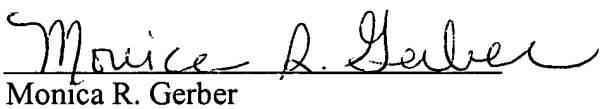
nucleic acid molecules containing 2-aminoadenosine provide stronger hybridization to a target sequence motivates their use in the claimed invention. The claimed invention does not involve hybridization to a target sequence. In fact it seeks to avoid intramolecular hybridization. In any event, claim 33 requires that the nucleic acid molecule contains 2-thiothymidine, inosine, and pyrrolopyrimidine *in addition to* 2-aminoadenosine, and claim 34 requires that the nucleic acid molecule contains 2-thiothymidine *in addition to* 2-aminoadenosine. Ross does not teach a nucleic acid molecule that contains these additional modified nucleotides. Neither does Lizardi. Therefore, there is no motivation to combine the teachings of Ross with those of Baldarelli or Lizardi, and even if such motivation existed, the combination would not result in the invention of claim 33 or 34. Withdrawal of the rejection is respectfully requested.

Claims 35 and 101 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Baldarelli in view of Lizardi as applied to claims 1-32, 67-75, and 78-100, and further in view of Thorp (5,871,918), hereinafter "Thorp". The Examiner points out that Thorp discloses a method of detecting a nucleic acid by use of tunneling and therefore asserts that the use of tunneling to detect a nucleic acid as taught in the instant claims would be obvious. As explained above, Baldarelli and Lizardi do not render claims 1-32, 67-75, or 78-100 obvious. In particular, Lizardi does not teach or suggest modified nucleotides that could be used for purposes of reducing secondary structure as required by claims 35 and 101 through their dependency on claims 1 and 67. The Examiner has not suggested that Thorp adds anything to the combination of Baldarelli and Lizardi with respect to the use of modified nucleotides to reduce secondary structure. Therefore, the combination of Baldarelli, Lizardi, and Thorp does not render claim 35 or claim 101 obvious. Withdrawal of the rejection is respectfully requested.

In conclusion, in view of the amendments and remarks presented herein, the application and pending claims comply with the requirements of 35 U.S.C. §101 and §112. Applicant therefore respectfully submits that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

Please charge the fee for a one (1) month extension of time, and any additional fees associated with this filing, or apply any credits, to Deposit Account No. 50-1078.

Respectfully submitted,



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